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(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING SYNTHETIC PEPTIDE COPOLYMER FOR PREVENTION OF GVHD

(57) Abstract

Pharmaceutical compositions comprising as active ingredient a synthetic random copolymer of average molecular weight of about 4,000-12,000, preferably 6,000-8,000, said copolymer consisting of glutamic acid (Glu), lysine (Lys), alanine (Ala) and tyrosine (Tyr) residues in a relative molar ratio of 1.4-2.1 parts of Glu to 3.2-5.0 parts of Lys to 4.0-6.6 parts of Ala to 1.0 parts of Tyr (herein GLAT copolymers), can be used in a method for prevention and treatment of graft-versus-host disease in patients in the course of bone marrow and organ transplantation.

PHARMACEUTICAL COMPOSITIONS COMPRISING SYNTHETIC PEPTIDE COPOLYMER FOR PREVENTION OF GVHD

FIELD OF THE INVENTION

The present invention relates to organ transplantation in humans in general, and more specifically to prevention of graft-versus-host-disease, particularly in bone marrow transplantation.

BACKGROUND OF THE INVENTION

Bone marrow transplantation (BMT) is increasingly used in humans for treatment of many life-threatening hematologic disorders, especially leukemia and certain immune deficiency diseases. However, graft-versus-host disease (GVHD) remains the major barrier for effective allogeneic bone marrow transplantation. This complication occurs in approximately 30% of bone marrow recipients. Up to half of those patients who develop GVHD may succumb to this process. This high morbidity and mortality has led to continuous interest in the possibility of controlling or preventing GVHD.

Current available approaches for prevention of GVHD include the use of non-specific immunosuppressive drugs, such as cyclosporine, methotrexate and/or prednisone. However, this treatment induces severe side effects, including nephrotoxicity, hypertension, hypercholesterolemia, diabetogenic effects, neurotoxicity, hirsutism and gengival hyperplasia. Moreover, the unselective depression of the entire immune system renders patients vulnerable to infections. In addition to the chronic administration of toxic, immunosuppressive agents, successful human allogeneic bone marrow transplantation depends, at the moment, on the availability of a suitable histocompatibility donor.

Clinicopathologically, two forms of GVHD have been recognized. Acute GVHD develops within the first 3 months after BMT and features disorders of skin, liver and gastrointestinal tract. Chronic GVHD is a multiorgan

autoimmune-like disease, emerging from 3 months up to 3 years post-transplantation and shares features common to naturally occurring autoimmune disorders, like systemic lupus erythematosus (SLE) and scleroderma.

GVHD is caused by the competent donor T cells reacting against minor histocompatibility antigens (mHAg) of the recipient, even when the donor and recipient are major histocompatibility complex (MHC)-identical. The donor T cells are sensitized to such alloantigens and then directly, or through secondary signals, attack the host cells. These mHAg display a tissue-specific distribution (Griem et al., 1991). Recent experimental evidence has clearly demonstrated that human (den Haan et al., 1995; Wang et al., 1995) and murine (Speiser, et al., 1990; Wallny et al., 1990; Roopenian, et al., 1993) mHAg correspond to recipient self-peptides presented by MHC class I and II molecules. This initial recognition process of host alloantigen after transplantation is further enhanced by the release of cytokines (Rus et al., 1995; Nestel et al., 1992) resulting in non-specific T cell activation and in the up-regulation and de novo expression of MHC molecules.

Novel experimental approaches towards prevention of murine GVHD have focused on the molecular basis of antigen recognition by T cells and on the early stages of T cell activation. These approaches aim at interference with the antigen-specific signal generated through the T cell receptor (Schlegel et al., 1994; Blazar et al., 1994) and at the inhibition of costimulatory signals (Schlegel et al., 1995). It has previously been demonstrated that small, synthetic peptides of 11-14 amino acids length with high binding affinity for specific class II MHC molecules are capable of preventing murine GVHD (Schlegel et al., 1994). This approach, however, has been limited by the need for allele-specificity of the inhibitor peptides (Schlegel et al., 1994) and by the difficulty of achieving sustained tissue levels of such low molecular weight peptides over a prolonged period of time after injection (Schlegel et al., 1994; Ishioka et al., 1994; Samson et al., 1995).

A high molecular weight synthetic basic random
copolymer consisting of L-Ala, L-Glu, L-Lys and L-Tyr residues
in the molar ratio of about 6 parts Ala to 2 parts Glu to 4.5
parts Lys to 1 part Tyr, and having a molecular weight of
15,000-25,000, was first described in US Patent No. 3,849,550
as an agent for treatment or prevention of experimental
allergic encephalomyelitis (EAE), a disease resembling
multiple sclerosis (MS) that can be induced in susceptible
animals (Sela et al., 1990; Teitelbaum et al., 1971;
Teitelbaum et al., 1973). Batches of this copolymer of
average molecular weight 23,000, designated Copolymer 1 or
Cop 1, were shown to be highly effective in protecting and
suppressing EAE in several animal species (Teitelbaum et al.,
1971, 1974a, 1974b). Later, Cop 1 was found to significantly
reduce the number of relapses in patients with the
exacerbating-remitting form of MS (Bornstein et al., 1990;
Johnson et al., 1994). Subsequent studies have demonstrated
the direct binding of Cop 1 to murine and human class II MHC
molecules of different haplotypes (Fridkis-Hareli et al.,
1994).

The mechanism underlying the therapeutic activity of
Cop 1 in MS has been extensively studied. The interaction of
Cop 1 with MHC molecules may result in two main and distinct
underlying mechanisms for disease suppression, namely the
induction of antigen specific suppressive regulatory cells
(Teitelbaum et al., 1988) and competition with myelin antigens
for activation of specific T cell responses (Fridkis-Hareli et
al., 1995). Recent studies demonstrated that Cop 1 binds
directly to class II molecules without the need for
intracellular processing (Claman et al., 1985).

The first step essential for the activation of these
specific processes is the binding of Cop 1 to the histo-
compatibility molecules. Indeed, it has been shown that a low
molecular weight version of Cop 1 - two different batches of
molecular weight 5,550 and 8,600, and relative molar ratio of
L-Ala (4.1-5.8 residues), L-Glu (1.4-1.8 residues), L-Lys
(3.2-4.2 residues) and L-Tyr (1 residue) - binds very
efficiently to a variety of MHC class II molecules of mouse

and human origin, and furthermore competes with myelin basic protein (MBP) and its major epitope p84-102 for MHC binding and can even displace such antigens that had already been bound to the MHC molecule (Fridkis-Hareli et al., 1994; WO 95/31990).

None of the prior art publications describes or suggests that any form of Cop 1 may be used to prevent or to treat GVHD.

SUMMARY OF THE INVENTION

It has now been found in accordance with the present invention that a GLAT copolymer can be used successfully to prevent experimental graft-versus-host disease in an animal model which is similar to the matched bone marrow transplantation in humans. This murine system comprises bone marrow and spleen cells of B10.D2 mice transplanted to BALB/c recipients (both H-2d), but clearly different in minor histocompatibility antigens. The murine GVHD model of B10.D2/nSnJ → BALB/c (both H-2d) across minor histocompatibility barriers has been described in detail (Schlegel et al., 1995; Korngold et al., 1987; Hamilton 1987; Webb et al., 1990) and was selected since it represents a model which is very similar to MHC-matched bone marrow transplantation in humans (Schlegel et al., 1995).

The present invention thus relates, in one aspect, to a pharmaceutical composition for use in the prevention and treatment of graft-versus-host disease comprising as active ingredient a synthetic random copolymer of average molecular weight of about 4,000-12,000 consisting of glutamic acid, lysine, alanine and tyrosine residues, in a relative molar ratio of 1.4-2.1 parts of Glu to 3.2-4.2 parts of Lys to 4.0-6.0 parts of Ala to 1.0 part of Tyr, herein referred to as GLAT copolymer, together with physiologically acceptable carriers.

The GLAT copolymer used in the present invention has preferably an average molecular weight of about 5,500-10,000 Da, more preferably of 6,000-8,000 Da, and most preferably, of about 6,000 or of about 8,000.

The Glu, Lys, Ala and Tyr residues may all have the L-configuration (L-GLAT) or the D configuration (D-GLAT) or some of the residues have the L, and the others the D, configuration (DL-GLAT). In one preferred embodiment, L-GLAT is used in the invention.

Preferred molar ratios of the amino acid residues include the relative molar ratios 1.9 Glu to 4.7 Lys to 6.0 Ala to 1.0 Tyr, 1.7 Glu to 3.8 Lys to 4.9 Ala to 1.0 Tyr, and 1.8 Glu to 4.0 Lys to 6.0 Ala to 1.0 Tyr.

Although we have described above some preferred embodiments of the invention, it is to be understood that the present invention encompasses any synthetic random copolymer of Glu, Lys, Ala and Tyr, having a relative molar ratio of the amino acid residues and an average molecular weight as defined herein, including those forms of Cop 1 described in the literature that fall within the definition of the present invention.

Another aspect the invention relates to the use of said GLAT copolymer for the manufacture of a medicament for prevention and treatment of graft-versus-host disease.

A further embodiment the invention relates to a method of treatment of a patient for prevention and treatment of graft-versus-host disease in the course of bone marrow and organ transplantation, said method comprising administering to said patient effective amounts of said GLAT copolymer.

In a preferred embodiment, the GLAT copolymer is used according to the invention for prevention of graft-versus-host disease in allogeneic bone marrow transplantation, optionally together with other immunosuppressive agents.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A-B show the inhibition of secondary mixed lymphocyte reaction (MLR) across minor as well as major histocompatibility barriers, caused by several peptides. The peptides L-GLAT (Batch I), MBP Ac1-11[4A], MBP 35-47, a mixture of MBP Ac1-11[4A] + MBP 35-47, MBP 89-101 and KM-core (all described in Materials and Methods hereinafter) were tested for their ability to inhibit the proliferation of

B10.PL (H-2^u) mice responder cells to stimulator cells of either the same haplotype (PL/J mice, Fig. 1A) or of a different H-2 haplotype (BALB/c mice, Fig. 1B).

Figs. 2A-E are graphs showing dose-dependent inhibition of the MLC across major histocompatibility barriers. (Figs. 2A,B,C,D H-2^d anti H-2^{u,s,k,b} and E, H-2^b anti H-2^d). 2.5 × 10^5 responder spleen cells were incubated with optional concentrations of irradiated (30 Gy, Cs source) spleen stimulator cells as described in Materials and Methods in a final volume of 200 μl. GLAT or HEL were added at the indicated concentrations (10-100 μg/well). After 96 hours of incubation, cultures were pulsed with 1 μCi [^{3}H]-thymidine for an additional 16 hours. Similar degrees of inhibition by GLAT were observed using various amounts of stimulator cells (2.5, 5.0 or 10 × 10^5, data not shown). No inhibition was observed with the addition of HEL in any of the strain combinations tested. Results represent one of five experiments with similar results.

Figs. 3A-D are graphs showing the effect of treatment with GLAT on the induction of GVHD in B10.D2/nSnJ → BALB/c recipients (□ L-GLAT (Batch III), n=25; o PBS, n=26; ▽ HEL, n=10). Fig. 3A shows onset of GVHD in individual mice of the experimental groups. The median disease onset is indicated by lines (day 73/GLAT, day 21/PBS, and day 22/HEL). Statistical analysis by x^2 distribution : Day 30 post-transplant : $p < 0.001$, incidence of GVHD in GLAT-treated mice (3/25) compared to the incidence in mice treated with PBS (26/26), or with HEL (10/10). Day 70 posttransplant : $p < 0.001$, incidence of GVHD in GLAT-treated mice (12/25) compared to PBS-treated (26/26), or $p < 0.02$, compared to HEL-treated mice (10/10). Fig. 3B shows maximum disease severity in individual mice of the experimental groups. The median disease severity is indicated by lines (2.0/GLAT, 5.5/PBS, 6.5/HEL). Fig. 3C shows actuarial survival from lethal GVHD. The median survival times (days) for the experimental groups are as follows: > 140 for GLAT, 47 for PBS, and 41 for HEL. Statistical analysis by Mann-Whitney test : $p < 0.01$, GLAT-treated mice compared to control mice

with either PBS or HEL. Fig. 3D shows mean body weight curves after transplant. Data given in Figs. 3A-D are pooled from three similar experiments.

Figs. 4A-B show the effect on survival of BALB/c mice transplanted with bone marrow and spleen cells from B10.D2 mice after treatment with L-GLAT (Batch I), chicken egg lysozyme (HEL) or phosphate-buffered saline (PBS) (Fig. 4A) or with L-GLAT (Batch II), HEL, PBS or TGA polymer (Fig. 4B).

Fig. 5 shows the effect on survival of BALB/c mice transplanted with bone marrow and spleen cells from B10.D2 mice after treatment with D-GLAT or PBS.

DETAILED DESCRIPTION OF THE INVENTION

The GLAT copolymers used in the present invention represent a novel therapeutic approach to treat human graft-versus-host disease for effective organ, particularly bone marrow, transplantation.

GLAT is a synthetic random basic copolymer analogous to Cop 1. Previous work has demonstrated two main and distinct mechanisms of action for Cop 1 which derive from its direct high-affinity binding to class II molecules of multiple murine and human alleles: a) cross-reactivity with myelin basic protein (MBP) at the humoral and cellular level leading to the induction of suppressive regulatory cells and b) competitive inhibition of the binding of myelin antigens to MHC, which may result in inhibition of specific effector T cell activation. The first mechanism has been shown to be most important for the prevention of EAE, a model in which a dominant antigen (MBP for $H-2^u$ and PLP for $H-2^s$ strains) leads to the induction phase of the autoimmune disease. On the contrary, GVHD is an alloimmune response induced by transplanted donor T cells in response to multiple minor antigens of the recipient. Some of the mHAg may occupy up to 10 % of binding sites whereas others may occupy as little as 0.001 % of MHC sites. Previous work has demonstrated that through the use of class-II binding allele-specific peptides of 11-14 amino acids, GVHD can be prevented. Prevention was found to be transient in some and permanent in others. This

approach, however, has been limited by the need for allele-
specificity and by the relatively short half-life of class-II
peptides. GLAT, being of larger molecular weight, has been
found to have an extended half-life in addition to its
promiscuous but specific binding properties for multiple MHC
class II alleles.

A series of *in vitro* experiments using T cell
proliferative responses to major and minor histocompatibility
antigens have shown that addition of GLAT inhibited the MLC in
a dose-dependent inhibition in a variety of systems ($H-2^d$ anti
$H-2^{u \cdot s \cdot k \cdot b}$ or $H-2^b$ anti $H-2^d$). Hen egg lysozyme (HEL) was
used as specificity control. To exclude any toxic effect of
GLAT on the responder cells, cells were successfully
rechallenged with IL-2 in a subsequent assay. Furthermore,
GLAT (1-100 μg/well) did not inhibit the IL-2 driven
proliferation of the murine T cell clone G81. To investigate
the role of T cell responses to minor antigens, a system was
designed that allowed for antigen-presentation of mHAg on
syngeneic antigen presenting cells (APC). GLAT reduced the
proliferative responses to mHAg in a dose-dependent fashion.
Maximum inhibition was found at a concentration of 80 μg/well.
In an additional experiment, APC were harvested, preincubated
for 24 hours with different concentrations of GLAT and washed
before incubation with the responder cells. Prepulsing of the
APC with GLAT reduced the subsequent proliferative response to
mHAg, suggesting that GLAT inhibits antigen-presentation.

Ligation of MHC class II molecules has recently been
shown to mediate apoptotic cell death in resting B lymphocytes
under certain conditions. Incubation of spleen cells with
GLAT for 48 or 72 hours did not result in apoptosis. These
results were confirmed by FACS analysis using the Hoechst dye
33342.

Data from three consecutive experiments *in vivo*
showed that administration of GLAT over a limited period after
transplant prevented GVHD across minor histocompatibility
barriers. Nine of 25 animals did not develop GVHD beyond the
observation period of 140 days post transplant, whereas all
control-treated mice (PBS or HEL) had developed GVHD by day

26. Furthermore, 12 of the GLAT treated mice showed a delayed
onset of GVHD (range 32-112). GLAT improved disease severity
as assessed by the severity score and by the mean body weight
curves. GLAT resulted in a significantly improved survival
from GVHD-related mortality (p<0.01) as compared to controls.
The selected system of GVHD closely resembles the clinical
setting of matched donor-recipient pairs. The murine system
of B10.D2/nSnJ → BALB/c has been well elaborated. Both CD4$^+$
and CD8$^+$ T cell subsets are responsible for GVHD-induction in
lethally irradiated BALB/c recipients, with CD4$^+$ T cells being
the predominant subset.

Data from the *in vitro* and *in vivo* studies are
consistent with the concept that GLAT, by virtue of its
ability to bind to multiple MHC class II molecules, inhibits
antigen-presentation. Administration of GLAT over a limited
time after transplant was successful in preventing the
development of GVHD. Thus, GLAT represents a novel class of
potent biological response modifiers for the prevention of
GVHD across minor histocompatibility barriers.

The L-GLAT to be used according to the present
invention may be prepared by methods known in the art, for
example, the process disclosed in US Patent 3,849,550 and in
Teitelbaum et al., 1971, wherein the N-carboxyanhydrides of
tyrosine, alanine, γ-benzyl glutamate and ε-N-
trifluoroacetyllysine are polymerized at ambient temperature
in anhydrous dioxane with diethylamine as initiator, followed
by deblocking of the γ-carboxyl group of the glutamic acid
with hydrogen bromide in glacial acetic acid, and removal of
the trifluoroacetyl groups from the lysine residues by 1M
piperidine. L-GLAT with the required molecular weight profil·
of 4,000-12,000, preferably 6,000-8,000, can be obtained by
methods known per se, for example, by chromatography of L-
GLAT-containing high molecular weight species or by partial
acid or enzymatic hydrolysis to remove the high molecular
weight species with subsequent purification by dialysis or
ultrafiltration.

D-Cop 1, an example of D-GLAT copolymer, has been
found as non-cross-reactive with either the L-copolymer,

Cop 1, or with the natural autoantigen myelin basic protein,
and failed to exhibit any suppressive activity on EAE,
indicating that it does not have a therapeutic value relevant
to MS (Webb et al., 1976). According to the present
invention, it was surprisingly found that D-GLAT binds as
efficiently as L-GLAT to the MHC molecules, indicating its
utility for prevention and treatment of GVHD.

D-GLAT and DL-GLAT copolymers may be prepared
similarly to L-GLAT copolymers using D and D+L
carboxyanhydrides, respectively, of the corresponding amino
acids. An advantage in using a D-GLAT or DL-GLAT copolymer
resides in the fact that such polymers metabolize much slower
than the L-isomer, and thus a longer half-life and higher
concentrations of the effective material can be achieved.

The pharmaceutical compositions of the present
invention comprising a GLAT copolymer are prepared by
conventional methods known in the art. Preferably, the
composition is lyophilized and formed into an aqueous solution
suitable for subcutaneous, intramuscular or intravenous
injection. Typically, a pharmaceutical composition will
comprise from 10 to 100 mg of L-GLAT in an unit dose. D-GLAT
and DL-GLAT copolymers may be used in lower doses.

The GLAT copolymer may be used according to the
invention for prevention and treatment of GVHD in all cases of
organ transplantation that develop GVHD, but particularly in
fetal thymus, and more particularly, in allogeneic bone
marrow transplantation. To a patient under suitable
conditioning regimen, the GLAT copolymer is administered from
day -2 prior to the transplantation day, and then for another
60-100, at least 60, days, after the transplantation day.
Other immunosuppressive drugs, such as cyclosporine,
methotrexate and prednisone, may be administered with the GLAT
copolymer.

The method of the invention is suitable for the
prevention and treatment of GVHD in the course of bone marrow
transplantation in patients suffering from diseases curable by
bone marrow transplantation, including leukemias, such as
acute lymphoblastic leukemia (ALL), acute nonlymphoblastic

leukemia (ANLL), acute myelocytic leukemia (AML) and chronic myelocytic leukemia (CML), severe combined immunodeficiency syndromes (SCID), osteopetrosis, aplastic anemia, Gaucher's disease, thalassemia and other congenital or genetically-determined hematopoietic or metabolic abnormalities.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

MATERIALS AND METHODS

(a)   Preparation of GLAT copolymers and controls

(i) L-GLAT was prepared by polymerization of the N-carboxyanhydrides of L-Ala, γ-benzyl-L-Glu, N,ε-trifluoroacetyl-L-Lys, and L-Tyr. The polymerization reaction was carried out at room temperature in anhydrous dioxane with diethylamine as initiator. Deblocking of the γ-carboxyl groups of the glutamic acid was carried out with hydrogen bromide in glacial acetic acid for 24 hours at room temperature, followed by removal of the trifluoroacetyl groups from the lysine residue by 1M piperidine. The end product is a mixture of acetate salts of random polypeptides with amino acid composition of Glu (1.4-2.1 residues), Lys (3.2-5.0 residues), Ala (4.0-6.6 residues), and Tyr (1 residue). Three L-GLAT batches were used in the experiments: Batch I consisting of a copolymer of molecular weight of about 6,000, with the amino acids in the molar ratio of about 1.7 Glu to 3.8 Lys to 4.9 Ala to 1.0 Tyr; Batch II, consisting of a copolymer of molecular weight of about 8,000, with the amino acids in the molar ratio of 1.8 Glu to 4.0 Lys to 6.0 Lys to 1.0 Tyr; and Batch III consisting of a copolymer of molecular weight of about 6,000 to 8,500 Da, with the amino acids in the molar ratio of 1.9 Glu to 4.7 Lys to 6.0 Ala to 1.0 Tyr.

(ii) D-GLAT was prepared by polymerization of the N-carboxyanhydrides of the D-amino acids D-Ala, γ-benzyl-D-Glu, N,ε-trifluoroacetyl-D-Lys, and D-Tyr in a residue molar ratio of 5.6 : 2.2 : 4.6 : 1.0 with an average molecular weight of approx. 29,000.

(iii) TGA is a random basic polymer of L-Tyr, γ-benzyl-L-Glu, and L-Ala in a residue molar ratio of 1.0: 1.2: 1.1. It was used as a negative control.

(iv) Hen egg-white lysozyme (HEL) was obtained from Sigma Chemical Company (St. Louis, MO).

(v) Peptides were synthesized by standard FMOC chemistry. All peptides were 95% to 99% pure, as determined by high-performance liquid chromatography, and were checked by amino acid analysis and mass spectroscopy. Sequences are given in three letter codes:

MBP Ac1-11[4A], an acetylated N-terminal 1-11 peptide of myelin basic protein (MBP), with substitution of the original Lys residue at position 4 by Ala: Ac-Ala-Ser-Gln-Ala-Arg-Pro-Ser-Gln-Arg-His-Gly (SEQ ID NO:1);

MBP 35-47, the epitope of MBP which is recognized in association with I-E^u: Thr-Gly-Ile-Leu-Asp-Ser-Ile-Gly-Arg-Phe-Phe-Ser-Gly (SEQ ID NO:2);

KM-core extension peptide, based on the antigenic core sequence of ovalbumin 323-339: Lys-Met-Lys-Met-Val-His-Ala-Ala-His-Ala-Lys-Met-Lys-Met (SEQ ID NO:3);

MBP 89-101: Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro (SEQ ID NO:4), was synthesized by t-butoxy-carbonyl chemistry.

(b) **Animals**

B10.D2/nSnJ (H-2^d), CBA (H-2^k), C57BL/6 (H-2^b), B10.PL (H-2^u) and PL/J (H-2^u) mice were purchased from The Jackson Laboratories (Bar Harbor, ME); BALB/c (H-2^d) recipient mice were obtained from Simonsen Laboratories (Gilroy, CA). B10.S (H-2^s) were obtained from Dr. H.O. McDevitt's colony and bred in the animal facility of Stanford University.

(c) **Mixed lymphocyte cultures (MLC)**

MLC were set up as previously described (Schlegel et al., 1995; Webb et al., 1990). Briefly, 2.5×10^5 responder spleen cells were plated in flat-bottom 96 well microculture plates with 2.5, 5.0 or 10×10^5 irradiated (30 Gy, Cs source) spleen stimulator cells in a final volume of 200 μl. GLAT or

relevant controls were added at the indicated concentrations
(10 - 100 μg/well). After 96 hours of incubation, cultures
were pulsed with 1 μCi [^{3}H]-thymidine for an additional 16
hours. Results are mean cpm from triplicate cultures.

(d) Assessment of GVHD

Mice were followed up daily for 140 days after bone
marrow transplantation for signs of GVHD. Disease severity
was assessed by mortality, loss of body weight, and by the
extent of macroscopic skin involvement scored on a cumulative
severity scale from 0 (minimum) to 8 (maximum): head 1, neck
1, back (1/3, 2/3, 3/3) 1-3, front (1/3, 2/3, 3/3) 1-3 as
previously described (Schlegel et al., 1994; Schlegel et al.,
1995; Claman et al., 1985; Korngold et al., 1987; Hamilton
1987).

(e) PCR analysis

Engraftment of donor bone marrow was documented by
PCR amplification of a polymorphic microsatellite region
within the murine IL-1β gene. Primer sequences are as
follows: 5'-CCAAGCTTCCTTGTGCAAGTA-3' (SEQ ID NO:5) and 5'-
AAGCCCAAAGTCCATCAGTGG-3' (SEQ ID NO:6) (Jacob et al., 1993).
These sequences are available from EMBL/Gen-Bank/DDBJ database
(Bethesda, MD) under the accession numbers: X78456 and
X78457. Oligonucleotides were synthesized on a 391 DNA
synthesizer (Applied Biosystems, Foster City, CA) and were
purified. DNA was prepared from peripheral blood mononuclear
cells 80-120 days after transplantation according to standard
protocols (Schlegel et al., 1994). PCR conditions and
amplification were as described previously (Schlegel et al.,
1994).

(f) Bone marrow transplant

For the induction of GVHD, 10 × 10^6 bone marrow and
100 × 10^6 spleen cells from B10.D2 mice were injected into
lethally irradiated (8.0 Gy) BALB/c recipients. This regimen
induces the most severe form of GVHD (Schlegel et al., 1995)
and was selected for all experiments of this study. Recipient

mice were 12 -13 weeks at the time of transplant. All
experiments were performed according to U.S. federal and
Stanford University guidelines.

(g) Treatment

Recipient BALB/c mice were treated with the
synthetic copolymer GLAT, phosphate-buffered saline (PBS), or
with HEL. Based on previous studies with Cop 1 in EAE (Sela
et al., 1990; Teitelbaum et al., 1971; Teitelbaum et al.,
1973) and class II-binding competitor peptides in GVHD
(Schlegel et al., 1994), the dosage of 600 μg per injection
was selected, half of which was administered intraperitoneally
(ip), whereas the other half was given subcutaneously (sc).
Treatment of BALB/c recipient mice was initiated on day -1,
followed by daily injections for the first five weeks starting
on day 0 after transplant. The frequency of injections was
tapered to three times per week for the subsequent two weeks
and to two times per week for another two weeks and then
discontinued. Throughout the experiment, copolymers and
controls were administered once a week with incomplete
Freund's adjuvant (IFA) ip as a depot dose (Schlegel et al.,
1994). Treatment was discontinued 9 weeks after transplant.

(h) Inhibition of minor histocompatibility (mH) antigen
     presentation

B10.D2 mice were injected i.p. with irradiated (20
Gy) BALB/c spleen cells 72 hours prior to the assay. B10.D2
spleen cells were harvested, depleted of RBC and used as
irradiated (10 Gy) APC presenting BALB/c mH antigens. APC
were plated at 2.5×10^5 cells/well in 96 well round-bottom
plates and incubated with 2.5×10^5 nylon-wool-enriched
responder T cells (> 88% CD3$^+$ by FACS analysis) from naive
B10.D2 mice in the presence or absence of increasing
concentrations of GLAT (2.5 - 80 μg/well). After 96 hours of
incubation, cells were pulsed with 1 μCi [^{3}H]-thymidine for an
additional 16 hours before harvesting. In a second
experiment, irradiated APC were plated at 2.5×10^5 cells/well
in 96 well round-bottom plates, preincubated for 24 hours at

37°C with increasing concentrations of GLAT (2.5-80 μg/well)
or with medium alone, and thereafter washed three times before
the addition of 2.5 × 10⁵ nylon-wool-enriched responder T
cells from naive B10.D2 mice. Culture medium was RPMI 1640,
supplemented with 10% pre-screened fetal calf serum, 2mM
glutamine, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, and
100 μg/ml streptomycin.

(i) Apoptosis

        B10.D2 spleen cells (1 × 10⁶ per ml) were cultured
in 24-well plates for 48 or 72 hours in the presence or
absence of GLAT or HEL at the indicated concentrations.
Irradiated (6.0Gy) B10.D2 thymocytes cultured for 24 hours
served as the positive control (Sellins et al., 1987). Cells
were collected, lysed (1mM EDTA, 50mM Tris (pH 7.5), 0.1 % NP-
40) with 0.5 mg of proteinase K. DNA was extracted with
phenol-chloroform two times and precipitated with ethanol
(Schlegel et al., 1994). Aliquots of DNA were transferred to
a 1.5 % agarose gel containing 0.05 μg/ml ethidium bromide.
Presence or absence of DNA fragmentation was assessed as
previously described (McConkey et al., 1988).

EXAMPLE 1: Inhibition of mixed lymphocyte reaction by L-GLAT
        Mixed lymphocyte reaction (MLR), the proliferative
response of allogeneic lymphocytes when cultured together, is
considered an *in vitro* model for the recognition phase of the
GVHD reaction, and is part of the routine screening for bone
marrow donors.
        To investigate the feasibility of inhibition of MLR
by L-GLAT, an MLR system was developed in which L-GLAT was
tested for its ability to inhibit the proliferation of B10.PL
(H-2ᵘ) responder cells to stimulator cells of either the same
(PL/J) or different (BALB/c) H-2 haplotype. For comparison,
the inhibitory MBP Ac 1-11[4A], MBP 35-47, a combination of Ac
1-11[4A] + MBP 35-47, MBP 89-101 and RM-core peptides,
described in Schlegel et al., 1994, were used in the MLR
experiments.

As shown in Fig. 1A for stimulator cells of PL/J
mice and in Fig. 1B for stimulator cells of BALB/c mice, L-
GLAT (Batch I) significantly inhibited MLR across minor as
well as major histocompatibility barriers.   63% and 77%
inhibition could be obtained, respectively, when 1:1 ratio of
responder to stimulator cells was used, while the MBP 89-101
and the KM-core peptides, which are specific to the H-2'
haplotype, did not induce any significant effect.   The
inhibition obtained by L-GLAT was similar (in response to
minor histocompatibility antigen) or even higher (in response
to major histocompatibility antigen) than the inhibition
obtained with the combination of the two synthetic peptides Ac
1-11[4A] and MBP 35-47, which specifically bind to the class
II molecules I-A^u and the I-E^u, respectively.   The molar
efficiency of L-GLAT of M.W. 6000 is even higher since the
molecular weight of the synthetic peptides is 4-5 fold lower.

**EXAMPLE 2:   Inhibition of MLC across major histocompatibility
              barriers**

The effect of the synthetic copolymer L-GLAT (Batch
III) on mixed lymphocyte cultures across major
histocompatibility barriers was tested.   T cell proliferation
was assessed in six different MHC-disparate strain
combinations.   Data given in Figs. 2A-E are representative of
five separate experiments with similar results.   In all
experiments, addition of L-GLAT (10-100 μg/well) resulted in a
dose-dependent inhibition of the MLC.   HEL showed no or only
minimal inhibitory effect at all concentrations tested (Figs.
2A-E).   10-25 μg/well of L-GLAT was sufficient to achieve 50%
inhibition of the proliferative responses.   Maximum inhibition
(100%) was obtained in all strain combinations tested.   To
exclude the possibility that higher concentrations of L-GLAT
(50-100 μg/well) might be toxic to the responder cells,
responder cells from background wells and from wells incubated
with 25-100 μg/well of L-GLAT in the presence of stimulator
cells for 72 hours were rechallenged in a subsequent assay
with interleukin-2.   As shown in Table 1, there was no
difference between the secondary proliferative responses of

the groups tested. Responder cells that had been incubated
with stimulator cells in the presence of GLAT for 72 hours
were equally responsive to IL-2 compared to non-treated
cells.

Table 1: Effect of incubation with different concentrations of GLAT on the subsequent proliferative response to re-stimulation with IL-2[¶].

Primary Responder	Assay[*] Stimulator	GLAT (µg/well)	cpm	Secondary Stimulus	Assay[¶] cpm
B10.D2	-	-	564	IL-2	9,170
B10.D2	C57BL/6	-	20,333	IL-2	8,305
B10.D2	C57BL/6	100 µg	283	IL-2	7,951
B10.D2	C57BL/6	50 µg	3,663	IL-2	15,123
B10.D2	C57BL/6	25 µg	15,953	IL-2	11,994

[*] Primary Assay (left): 2.5×10^5 responder spleen cells were incubated with 5×10^5 irradiated (30Gy) spleen stimulator cells in a final volume of 200 µl in the presence or absence of L-GLAT (Batch III) as indicated. After 96 hours of incubation, cultures were pulsed with 1 µCi [³H]-thymidine for an additional 16 hours.

[¶] Secondary assay (right): Identical cultures were set up in parallel to the primary assay. After 72 hours of incubation, responder cells were re-challenged with interleukin-2 (5,000 U/well) for an additional 48 hours before pulsing with 1 µCi [³H]-thymidine for an additional 16 hours. Results are expressed as mean cpm from triplicate cultures. Standard deviations were less < 16% of the mean.

**EXAMPLE 3:   Inhibition of proliferative responses to minor H
                antigens *in vitro***

        Nylon wool-enriched T cells (>88% CD3⁺ cells by FACS
analysis) from naive B10.D2 were incubated for 96 hours with
syngeneic APC presenting minor BALB/c antigens as described in
Materials and Methods. As shown in Table 2, L-GLAT (Batch
III) inhibited the proliferative responses to mH antigens by
in a dose-dependent fashion. Maximum inhibition was 100% at a
concentration of 80 μg/well. To further elucidate the
mechanism of inhibition, APC were preincubated for 24 hours
with increasing concentrations of L-GLAT (2.5-80 μg/well),
washed and plated thereafter with responder T cells. As shown
in Table 2, preincubation with GLAT inhibited proliferative
responses to minor H antigens, suggesting that GLAT inhibits
presentation of minor antigens *in vitro*.

Table 2: Dose-dependent inhibition of proliferative responses to minor H antigens¶

Resp.	APC	GLAT assay concentration	cpm
+	-	-	10,464
+	+	-	108,574
+	+	80 µg	361
+	+	40 µg	23,591
+	+	20 µg	75,840
+	+	10 µg	119,866
+	+	5 µg	126,405
+	+	1.5 µg	124,904

Resp.	APC	Pre-pulsed APC	GLAT pulsing concentration	cpm
+	-	-	-	7,855
+	+	-	-	16,724
+	-	+	80 µg	4,628
+	-	+	40 µg	7,653
+	-	-	20 µg	10,465
+	-	+	10 µg	11,425
+	-	+	5 µg	12,377
+	-	+	2.5 µg	15,533

¶ Left: B10.D2 mice were injected i.p. with irradiated (20Gy) BALB/c spleen cells 72 hours prior to the assay. B10.D2 spleen cells were harvested, depleted of RBC and used as irradiated (10Gy) APC presenting BALB/c mH antigens. APC were plated at 2.5 x 10⁵ cells/well in 96 well round-bottom plates and incubated with 2.5 x 10⁵ nylon-wool-enriched responder T cells (> 88% CD3⁺ by FACS analysis) from naive B10.D2 mice in the presence or absence of increasing concentrations of L-GLAT (Batch III) (2.5-80µg/well). After 96 hours of incubation, cells were pulsed with 1 µCi [³H]-thymidine for an additional 16 hours before harvesting. Right: In a second experiment, irradiated (20Gy) APC were plated at 2.5 x 10⁵ cells/well in 96 well round-bottom plates, preincubated for 24 hours at 37°C with increasing concentrations of L-GLAT (Batch III) (2.5-80 µg/well) or with medium alone, and thereafter washed three times before the addition of 2.5 x 10⁵ nylon wool-enriched responder T cells from naive B10.D2 mice. Results are expressed as cpm from triplicate cultures. Typical standard deviations were < 20% of the mean. Culture medium was RPMI 1640, supplemented with 10% pre-screened fetal calf serum, 2mM glutamine, 50 µM 2-mercaptoethanol, 100 U/ml penicillin, and 100 µg/ml streptomycin.

EXAMPLE 4:   Absence of apoptosis

Ligation of MHC class II molecules has recently been
shown to mediate apoptotic cell death in resting B lymphocytes
(Newell et al., 1993). To determine whether GLAT might induce
apoptosis in class-II bearing cells (B cells and/or T cells)
murine spleen cells were incubated with increasing
concentrations of L-GLAT (Batch III) or HEL-control for 48 or
72 hours. Aliquots of cells were processed for DNA extraction
and DNA was visualized by gel electrophoresis. L-GLAT did not
induce apoptosis at any of the concentrations tested.

**EXAMPLE 5:   Bone marrow transplantation in mice, induction of
              GVHD and treatment with L-GLAT**

(i)   Initial titration study. For the induction of
GVHD across minor histocompatibility barriers, an initial
titration study was performed by transplanting 10×10^6 B10.D2
bone marrow cells and increasing amounts ($10-100 \times 10^6$) of
B10.D2 spleen cells into lethally irradiated (8.0 Gy) 12-13
week old BALB/c recipients. The regimen of infusing 10×10^6
bone marrow cells and 100×10^6 spleen cells resulted in the
most severe form of GVHD, and was selected for all subsequent
experiments.

(ii)   Effect of L-GLAT treatment on the incidence,
onset and severity of GVHD. Recipient mice were pretreated
with 600 μg of L-GLAT (Batch III) or with the respective
controls (PBS, HEL) on day -1. For the first five weeks after
transplant, mice were injected daily as outlined in Materials
and Methods, followed by a tapering schedule over an
additional four weeks. Data from three consecutive
experiments are summarized in Figures 3A-D. Administration of
L-GLAT significantly reduced the overall incidence of GVHD (as
determined by typical skin changes and weight loss) from 100%
(26/26, 10/10) in control mice to 12% (3/25) (p<0.001) in L-
GLAT-treated animals on day 30 after transplant, and from 100%
in controls to 12/25 (48%) on day 70 after transplant
(p<0.02). Figure 3A depicts the onset of GVHD in individual
mice of the different experimental groups. In 12/25 animals
treated with L-GLAT the onset of GVHD was delayed with a range

of 32-112 days after transplant (median of 73 days) as
compared to control mice treated with either PBS (median onset
of 21 days) or HEL (median onset 22 days). Nine of 25 animals
treated with the copolymer L-GLAT did not develop any signs of
GVHD beyond the observation period of 140 days after
transplant (Fig. 3A). Furthermore, treatment with GLAT
improved overall disease severity as gauged by the disease
severity score (Fig. 3B) and by mean body weight curves of
transplanted animals (Fig. 3D).

Similar results were obtained with Batches I and II
of L-GLAT.

(iii)   _Effect of L-GLAT treatment on survival_.
Treatment with L-GLAT (Batch III) improved long-term survival
from lethal graft-versus-host disease. As shown in Fig. 3C,
14/25 (56%) of experimental mice survived more than 140 days
after transplant as compared to 2/26 of PBS treated or to 1/10
of HEL treated control mice (p<0.01). Treatment with HEL did
not improve long-term survival.

Similar experiments were performed substituting L-
GLAT Batch I (Fig. 4A) or Batch II (Fig. 4B). As shown in
Figs. 4A-B, treatment with L-GLAT for the first nine weeks
after bone marrow transplantation improved long-term survival
from lethal GVHD. Fig. 4A shows that 9 out of 10 (90%)
experimental mice survived more than 140 days after transplant
as compared to only 1 out of 10 for the PBS control or 1 out
of 6 for the HEL-treated control mice. In these figures,
means are stated as overall means of the respective groups.
GVHD-related mortality. MST: median survival time (in days).
Statistical analysis: Incidence of GVHD by x^2 distribution:
Experiment 1 (Fig. 4A): * p < 0.01, ** p < 0.001, L-GLAT
compared to PBS or HEL. Experiment 2 (Fig. 4B): * p <
0.02, L-GLAT compared to PBS, TGA or HEL.

Similar results were obtained with L-GLAT Batch II,
as shown in Fig. 4B.

(iv)   _Documentation of engraftment_. PCR analysis
was performed 100 days post-transplant as described in
Materials and Methods, section (e), to document long-term
engraftment of allogeneic bone marrow cells. DNA polymorphism

based on length variation in tandem repeat sequences of a
microsatellite in the murine IL-1 gene was used as marker to
differentiate between donor-derived (B10.D2/nSnJ) and
recipient (BALB/c) peripheral blood mononuclear cells. Long-
term engraftment of donor-derived cells, i.e., complete
chimerism, was demonstrated in allogeneic mice by PCR analysis
irrespective of the treatment received.

**EXAMPLE 6: Bone marrow transplantation in mice, induction of
GVHD and treatment with D-GLAT**

    (i)   Initial titration study was carried out as in
Example 5(i).

    (ii)   Effect of D-GLAT treatment on the incidence,
onset and severity of GVHD. Recipient mice were treated with
60 μg of D-GLAT or PBS daily for the first five weeks after
transplant followed by a tapering schedule over an additional
four weeks. The dosage of 60 μg/injection was selected based
on the prolonged half-life of D-GLAT and was administered
intraperitoneally (ip). Treatment was initiated on day -1 and
after five weeks the frequency of injections was tapered to
three times per week for the following two weeks and to two
times per week for another two weeks. Once a week the
copolymer D-GLAT was administered with incomplete Freund's
adjuvant (IFA) ip as a depot dose. Treatment was discontinued
after 9 weeks.

    The results are shown in Table 3. D-GLAT treatment
reduced the overall incidence of GVHD after allogeneic bone
marrow transplantation from 100% (7/7) in control mice to 43%
(3/7) in D-GLAT-treated animals on day 30. Two of seven
animals treated with D-GLAT did not develop any signs of GVHD
beyond the observation period of 140 days after transplant.
Furthermore, treatment with D-GLAT improved overall disease
severity as gauged by the mean disease severity score (Table
3).

(iii) Effect of D-GLAT treatment on survival.

Treatment with D-GLAT improved long-term survival from lethal
graft-versus-host disease. As shown in Fig. 3, 5/7 (71.4%) of
experimental mice survived more than 140 days after transplant
as compared to 0/7 of PBS-treated control mice.

Table 3: Effect of treatment with D-GLAT on the induction of GVHD in B10.D2 - BALB/c Recipients (Experiment 6)

Group	n	Incidence day 30	Incid. day 70	Incid. day 100	Incid. day 140	Mean Onset (days)	Mean Severity¶	MST (days)	% Actuarial Survival (day)
D-GLAT	7	3/7	5/7	5/7	5/7	34.8	2.1	>140	71.4 (140)
PBS	7	7/7	7/7	††	††	21.9	5.6	60.5	0 (96)

¶ Means are stated as overall means of the respective groups.
†† GVHD-related mortality. MST: median survival time (in days).

EXAMPLE 7: Prevention of GVHD in humans

(i) Patients. A protocol is established for patients aged 60 years or less and eligible for allogeneic bone marrow transplantation from histocompatible sibling donors for acute non-lymphoblastic leukemia or acute lymphoblastic leukemia not in first remission, chronic myelogenous leukemia not in chronic phase or relapsed patients with non-Hodgkin's lymphoma. Without bone marrow transplantation, these patients have expected survival measured in months. The benefits for these subjects is the use of high dose therapy in curing their disease and the potential prophylaxis against morbidity associated with allogeneic bone marrow transplantation, such as GVHD.

(ii) Conditioning Regimen. Patients are submitted to a conditioning regimen comprising fractionated total body irradiation by administering 120 cGy per fraction 2 to 3 times daily over 4 days (days -8 to -5) to a total dose of 1200 cGy, a multiple drug treatment including, e.g., etoposide (60 mg/kg) over 4 hours at day -4, cyclophosphamide (60 mg/kg) over 1 hour at day -2 , antibacterial agents, and standard prophylaxis agents against GVHD, e.g., cyclosporine, methotrexate and prednisone.

(iii) GVHD prophylaxis. GLAT copolymer is administered to the patient subcutaneously (sc), intramuscularly (im) or intravenously (iv) as a twice daily injection at a dosage of 1-500 mg twice daily. The treatment starts at day -2 before and is continued until day +60 after the allogeneic bone marrow transplantation (BMT). This day is chosen since nearly all of the acute GVHD that may occur, will do so by day +40.

Standard prophylaxis against GVHD with cyclosporine and prednisone is also continued. Cyclosporine is administered until the patient is able to sustain oral caloric intake and has no evidence of gastrointestinal toxicity (usually around the end of the first month, 4th week following BMT), at a dose from 1.5 to 5 mg/kg iv twice daily, by infusion in the first 35 days and then orally (per os) until the end of the treatment (day +180). Serum samples are

obtained and, if necessary, the drug concentration is adjusted to prevent drug-related toxicities. The aim is for a level of cyclosporine between 200-500 ng/ml. Methylprednisolone is administered iv until patients can be switched to oral (p.o.) prednisone. For example, it is administered iv at a dose of 0.25 mg/kg - 0.5 mg/kg from day +7 to +28 and p.o. at a dose of 0.4 mg/kg - 0.1 mg/kg until the end of the treatment (day +180).

The first phase of the treatment is to establish engraftment. If engraftment is established, the post-transplantation immunosuppression is then stepwise decreased. The first step is to stop the use of prednisone. If no GVHD occurs, then the use of cyclosporine is stopped (see statistical analysis). At that time only the GLAT copolymer is used as the immunosuppressive regimen.

(iv) Statistical analysis. Current engraftment success rate of bone marrow is close to 100% with a rate of about 50% at 20 days for patients satisfying the eligibility criteria of this protocol. Among these patients, the rate of relapse depends on the disease and remission status. The time to relapse curve is well approximated by an exponential curve over this interval. Both the engraftment rate at 20 days and the relapse rate are monitored statistically as the data become available on other patients treated by the same protocol.

The engraftment rate at 20 days provides the basis for stopping early to avoid putting more patients at risk than necessary if the stem cells take longer to engraft than anticipated. The binary endpoint of success by the 20th day is monitored by sequential test, using 20 as the hypothesized median based on past experience, and using a boundary for inferiority fixed to provide a Type I risk of 5% (one sided) and a degree of conservatism midway between constant p level and the O'Brien-Fleming approach.

As the treatment proceeds, the data on relapse is also monitored, using the time to relapse as the endpoint and including all patients under treatment, whether engraftment is successful at the 20th day or not.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

The present application is a continuation-in-part of U.S. application 08/540,388, filed 06 October 1995, now pending, and a continuation-in-part of U.S. application 08/421,412, filed 14 April 1995, now pending, the entire contents of both of which being hereby incorporated herein by reference.

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32

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 11 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) FEATURE INFORMATION: The alanine at position 1 is acetylated

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

    Ala Ser Gln Ala Arg Pro Ser Gln Arg His Gly
    1   5   10

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 13 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

    Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Ser Gly
    1   5   10

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 14 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

    Lys Met Lys Met Val His Ala Ala His Ala Lys Met Lys Met
    1   5   10

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 13 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

    Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro
    1   5   10

33

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCAAGCTTCC TTGTGCAAGT A   21

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGCCCAAAGT CCATCAGTGG   20

CLAIMS

1. A pharmaceutical composition for use in the prevention and treatment of graft-versus-host disease comprising a pharmaceutically acceptable carrier and, as active ingredient, a synthetic random copolymer of average molecular weight 4,000-12,000, herein referred to as GLAT copolymer, said GLAT copolymer consisting of glutamic acid (Glu), lysine (Lys), alanine (Ala) and tyrosine (Tyr) residues in a relative molar ratio of 1.4-2.1 parts of Glu to 3.2-5.0 parts of Lys to 4.0-6.6 parts of Ala to 1.0 part of Tyr.

2. A pharmaceutical composition according to claim 1, wherein the GLAT copolymer has an average molecular weight of about 5,500-10,000.

3. A pharmaceutical composition according to claim 1, wherein the GLAT copolymer has an average molecular weight of about 6,000-8,000.

4. A pharmaceutical composition according to any one of claims 1-3, wherein all amino acid residues in the copolymer have the L configuration, herein referred to as L-GLAT copolymer.

5. A pharmaceutical composition according to any one of claims 1-3, wherein all amino acid residues in the copolymer have the D configuration, herein referred to as D-GLAT copolymer.

6. A pharmaceutical composition according to any one of claims 1-3, wherein some of the amino acid residues of the copolymer have the L configuration and the other amino acid residues have the D configuration, herein referred to as DL-GLAT copolymer.

7.   A pharmaceutical composition according to claim
4, wherein said L-GLAT copolymer has an average molecular
weight of about 6,000 and a relative molar ratio of 1.7 parts
Glu to 3.8 parts Lys to 4.9 parts Ala to 1.0 parts Tyr.

8.   A pharmaceutical composition according to claim
4, wherein said L-GLAT copolymer has an average molecular
weight of about 8,000 and a relative molar ratio of 1.8 parts
Glu to 4.0 parts Lys to 6.0 parts Ala to 1.0 parts Tyr.

9.   A pharmaceutical composition according to claim
4, wherein said L-GLAT copolymer has a relative molar ratio of
1.9 Glu to 4.7 Lys to 6.0 Ala to 1.0 Tyr.

10.   A pharmaceutical composition according to claim
1 to be administered as intravenous, intramuscular or
subcutaneous injection.

11.   A method for preventing and treating graft-
versus-host disease in a patient in the course of bone marrow
or organ transplantation, which comprises administering to
said patient effective amounts of a synthetic random copolymer
of average molecular weight 4,000-12,000, herein referred to
as GLAT copolymer, said GLAT copolymer consisting of glutamic
acid (Glu), lysine (Lys), alanine (Ala) and tyrosine (Tyr)
residues in a relative molar ratio of 1.4-2.1 parts of Glu to
3.2-5.0 parts of Lys to 4.0-6.6 parts of Ala to 1.0 part of
Tyr.

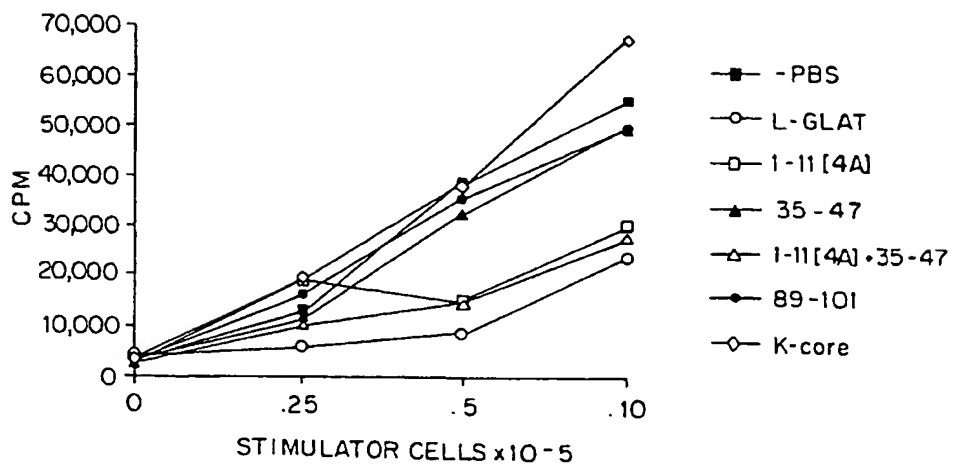12.   A method according to claim 11, wherein the
GLAT copolymer has an average molecular weight of about 5,500-
10,000.

13.   A method according to claim 11, wherein the
GLAT copolymer has an average molecular weight of about 6,000-
8,000.

14. A method according to any one of claims 11-13, wherein all amino acid residues in the copolymer have the L configuration, herein referred to as L-GLAT copolymer.

15. A method according to claim 14, wherein said L-GLAT copolymer has an average molecular weight of about 6,000 and a relative molar ratio of 1.7 parts Glu to 3.8 parts Lys to 4.9 parts Ala to 1.0 Tyr.

16. A method according to claim 14, wherein said L-GLAT copolymer has an average molecular weight of about 8,000 and a relative molar ratio of 1.8 parts Glu to 4.0 parts Lys to 6.0 parts Ala to 1.0 parts Tyr.

17. A method according to claim 14, wherein said L-GLAT copolymer has a relative molar ratio of 1.9 parts Glu to 4.7 parts Lys to 6.0 parts Ala to 1.0 parts Tyr.

18. A method according to any one of claims 11-13, wherein all amino acid residues in the copolymer have the D configuration, herein referred to as D-GLAT copolymer.

19. A method according to any one of claims 11-13, wherein some of the amino acid residues of the copolymer have the L configuration and the other amino acid residues have the D configuration, herein referred to as DL-GLAT copolymer .

20. A synthetic random copolymer of average molecular weight 4,000-12,000, consisting of glutamic acid (Glu), lysine (Lys), alanine (Ala) and tyrosine (Tyr) residues in a relative molar ratio of 1.4-2.1 parts of Glu to 3.2-5.0 parts of Lys to 4.0-6.6 parts of Ala to 1.0 part of Tyr.

FIG. 1A

FIG. 1B

FIG.2A
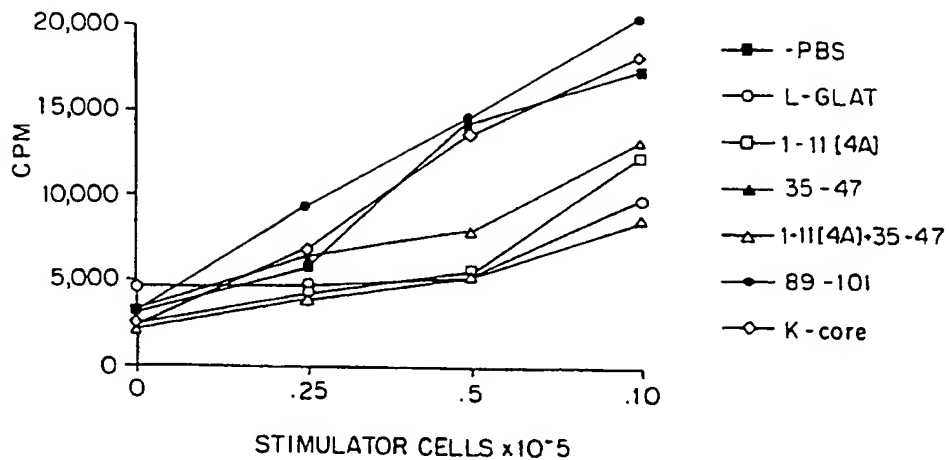
FIG.2B

FIG.2C

FIG.2D

FIG.2E
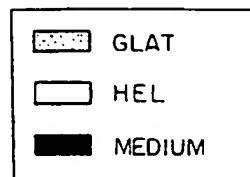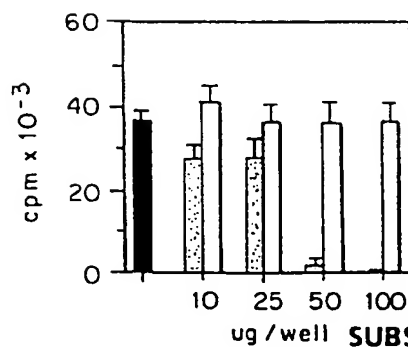
SUBSTITUTE SHEET (RULE 26)

FIG.3A
FIG.3B
FIG.3C
FIG.3D

FIG. 4A

FIG. 4B

FIG.5

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61K31/785   C08G69/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61K   C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BLOOD 84 (8). 1994. 2802-2810, XP000577169 SCHLEGEL P G ET AL: "Prevention of graft - versus - host disease by peptide binding to class II major histocompatibility complex molecules." cited in the application see the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 1996	07. 08. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

Inter   onal Application No
PC /US 96/05215

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 91 (11). 1994. 4872-4876, XP002009925 FRIDKIS-HARELI M ET AL: "Direct binding of myelin basic protein and synthetic copolymer 1 to class II major histocompatibility complex molecules on living antigen-presenting cells-specificity and promiscuity." cited in the application see the whole document ---	1-20
A	US,A,3 849 550 (TEITELBAUM D. ET AL.) 19 November 1974 cited in the application see the whole document ---	1-20
P,X	37TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HEMATOLOGY, SEATTLE, WASHINGTON, USA, DECEMBER 1-5, 1995. BLOOD 86 (10 SUPPL. 1). 1995. 224A, XP000578001 SCHLEGEL P G ET AL: "Inhibition of allorecognition and prevention of graft-vs-host disease (GVHD) by GLAT, a synthetic polymer with promiscuous binding to murine and human MHC class II molecules." see the whole document ---	1-20
P,X	WO,A,95 31990 (YEDA RESEARCH AND DEVELOPMENT) 30 November 1995 cited in the application see the whole document -----	20

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

page 2 of 2

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark:Although claims 11-19 are directed to a method of treatment of the
   human/animal body the search has been carried out and based on the alleged
   effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all
   searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-3849550	19-11-74	CA-A-	1006508	08-03-77
		CH-A-	570167	15-12-75
		DE-A-	2217817	16-11-72
		FR-A,B	2133926	01-12-72
		GB-A-	1393435	07-05-75
WO-A-9531990	30-11-95	AU-B-	2602195	18-12-95